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Title: Antimicrobial molecules in the lung: Formulation challenges and future directions for innovation

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Abstract

Inhaled antimicrobials have been extremely beneficial in treating respiratory infections, particularly chronic infections in a lung with cystic fibrosis (CF). The pulmonary delivery of antibiotics has been demonstrated to improve treatment efficacy, reduce systemic side-effects and, critically, potentially reduce drug exposure to commensal bacteria compared to systemic administration, reducing selective pressure for antimicrobial resistance (AMR). A number of chemical classes of antibiotic have been developed for inhaled drug delivery in nebulised and dry powder formulations (TOBI®, Cayston®, TOBI® Podhaler®, Colobreathe®), demonstrating the flexibility of this route for the administration of different therapeutic agents.

This review will explore the specific challenges of pulmonary delivery of a number of differing antimicrobial molecules, and the formulation and technological approaches that have been used to overcome these. It will also explore the future challenges being faced in the development of inhaled products and respiratory infection treatment, and identify future directions of innovation, with a particular focus on the development of urgently needed novel antimicrobials for the treatment of multi-drug resistant (MDR) pathogens in respiratory infections.

Executive Summary

Rationale for inhaled antibiotics in treating respiratory infection

- Delivering antibiotics via inhalation can achieve higher local concentrations in the lung than systemic administration.
- Treatment outcomes, as well as the tolerability of the treatment, can be improved as systemic concentrations are lowered, reducing the risk of treatment-associated side effects.

Antibiotic chemical classes in current use

- Inhaled solution and/or dry-powder formulations of tobramycin, colistin, aztreonam and levofloxacin have been approved for the treatment of *Pseudomonas aeruginosa* infections in a lung with cystic fibrosis , and a number of other classes have been reported in development
- The physicochemical properties of the antibiotic molecule (lipophilicity, logP, aqueous solubility, stability in airway lining fluid) affect the suitability of the drug for inhaled application, with the key requirement being to achieve and sustain high drug concentrations (> mutant prevention concentration, MPC)

Future directions

- Engineering of advanced carriers in the micron and nano-scale can facilitate modulation of drug PK in the lung and improve bacterial cell targeting following inhalation, and thus can improve the efficacy of inhaled antibiotics compared to free drug molecules.
- As the search for desperately needed novel antimicrobials continues, the formulation of these molecules for inhaled delivery should be taken seriously, to enable their use for

highly targeted and efficient treatment of deadly multi-drug resistant bacteria in respiratory infections.

Contents

1. Introduction

Respiratory tract infections represent one of the greatest threats to global health. The 2015 "Global Burden of Disease" study [1] identified that lower respiratory tract infections were the cause of 2.7 million deaths worldwide, of which 704,000 were in children under the age of 5 years old representing 12.1% of mortalities in this age group.

The rapid increase in antimicrobial resistance (AMR) observed since the introduction of penicillin to the market in 1942 is well documented, with ever increasing numbers of pathogens showing resistance to one or more antimicrobial drugs [2]. There has been an increase in the emergence of multidrug resistant (MDR) pathogens (resistant to ≥3 antimicrobial classes) in respiratory infections, including ventilator-associated and hospital-acquired pneumonia [3], tuberculosis [4] and cystic fibrosis-associated lung infections [5], which significantly increases the challenge of treating these deadly infections. A recent *in vitro* analysis of clinical isolates of *PA* obtained from CF patients adapted to long-term antimicrobial therapy showed that ~40% of strains were clinically resistant to at least one antibiotic tested and ~15% were multi-drug resistant [5]. All strains displayed evidence of mutations in genes known to be involved in mechanisms of antimicrobial resistance (e.g. *gyrA*, the target for fluoroquinolones; *mex* genes encoding efflux pump systems). In HAP/VAP, multidrug resistance is prevalent in many bacterial species including *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter* spp [3].

Systemic antimicrobial therapy through either the intravenous or oral route has traditionally been employed as first-line treatment for respiratory infection. However, ensuring sufficient penetration from the systemic circulation into lung tissue and infective foci to exceed the minimum inhibitory concentration (MIC) has proved to be challenging due to the limited absorption of many antimicrobials across the lung epithelium [6]. By contrast, inhaled drug delivery offers the opportunity to achieve high doses of drug directly at the site of infection, without requiring potentially toxic high systemic drug concentrations [7], and as a result this has been the subject of extensive research and development for application in lung infections.

2. Rationale for inhaled antibiotics

2.1 Achieving high doses at site of infection

In antimicrobial therapy, it is vital to employ the "right drug, right time, right dose" [8]. Using too low or too high a dose and/or incorrect dosing intervals can result in ineffective therapy, thus contributing to the emergence of antimicrobial resistance [8]. The common approach when assessing the success of antimicrobial therapy has been to ensure that the minimum inhibitory concentration (MIC), the minimum concentration which has been demonstrated to inhibit the growth of ~10^5 cfu/mL, has been reached [9]. Now however, understanding of the significance of the mutant prevention concentration (MPC) and the mutant selection window (MSW) informs the need to provide doses of antibiotic therapy >>MIC at an infection site. The mutant prevention concentration (MPC) is defined as the drug concentration at which an organism would need to possess two mutations in order for growth to not be inhibited, and thus is also equivalent to the concentration which would prevent the growth of first-step resistant cells [10]. Above the MPC, the growth of both resistant and non-resistant organisms will be inhibited. Below the MIC, neither resistant nor non-resistant organisms will experience significant growth inhibition and thus

antimicrobial therapy will fail. Between these concentrations lies the mutant selection window (MSW). In this concentration range, inhibition of non-resistant strains will occur, however there will also be a selective pressure in favour of proliferation of resistant organisms [10]. Thus, the proportion of resistant organisms within the bacterial community increases, increasing the concentration required to eradicate the infection and also increasing the risk of spreading these resistant strains from patient to patient. Low antibiotic concentrations can also induce the growth of hard to eradicate biofilm phenotype organisms [11]. There is therefore a strong clinical rationale when treating infection to achieve as high concentrations as can be tolerated at the site of infection, and to sustain concentrations above the MPC and MSW as much as possible.

Aerosolized antibiotics administered to the lung by inhalation have been demonstrated to achieve concentrations ≥MPC *in sputum* [12], something which can prove challenging with systemic delivery. For example, amikacin administration through inhalation has been demonstrated to result in lung concentrations which are 3-30 fold higher than that measured following intravenous administration [13], showing a clear benefit for this route of administration in achieving drug levels with reduced AMR-selective pressure.

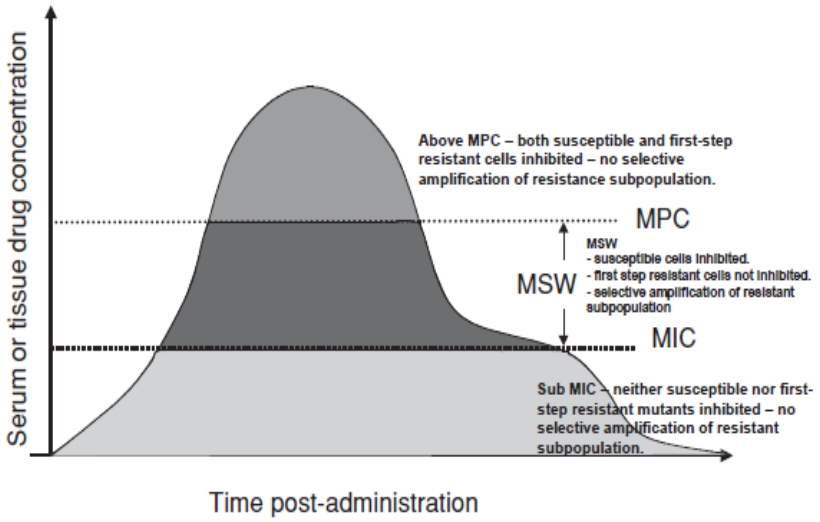


Figure 1. The mutant selection window. The success of antimicrobial therapy depends on the dose administered and the time at which that dose is sustained. In respiratory infection, lung drug concentrations must exceed both the minimum inhibitory concentration (MIC) and the mutant prevention concentration (MPC). Concentrations within this range risk selective amplification of resistant organisms. Figure reproduced from Blondeau *et al* (2004)

2.2 Reduced side effects

An additional benefit of inhaled antimicrobial therapy is that the delivery of drug directly to the airways removes the need for unnecessarily high serum concentrations. This is of particular importance when considering the serious adverse effects associated with many antibiotic classes used in the treatment of respiratory infections.

Intravenously administered aminoglycosides, such as tobramycin and gentamycin, form part of the recommended treatment regime for respiratory infection in cystic fibrosis [14]. However, the long-term use of aminoglycosides has been associated with dangerous side effects, including

effects on kidney function (nephrotoxicity) and the ear (ototoxicity) [15]. Nephrotoxicity can be monitored during aminoglycoside treatment by serum creatine levels, an increase indicating impaired clearance and thus renal function, and ototoxicity monitored by an audiogram assessment of patient hearing before and after treatment [16].

In CF patients, the effects of aminoglycosides are dose-limiting, and nephrotoxic levels are close to the therapeutic serum concentrations required [17]. The use of inhaled therapies can limit the serum concentrations required, thus reducing systemic exposure and the risk of associated side effects.

2.3 Reduction in exposure to gut microbiota

Oral fluoroquinolones, e.g. ciprofloxacin, levofloxacin, have been employed as well-tolerated and effective therapies for a number of respiratory infections, with the added benefits of being cheaper, more convenient and less invasive than intravenously administered antibiotic therapies [18,19]. However, the oral administration of antibiotics can have profound unwanted effects on the host's microbiota, particularly within the gut, including increasing selective pressure for AMR.

Administration of 500 mg ciprofloxacin twice daily for 5 days was demonstrated to reduce the richness, diversity and evenness of the host gut microbiota in 3 healthy volunteers as determined by 16S RNA sequencing [20]. The microbiota was determined to recover 4 weeks following the end of treatment. However, in the case of chronic infections in CF patients, therapy may be ongoing for months or even years, thus having long lasting effects on the gut flora. Long term fluoroquinolone use and the accompanying reduction in microbial biodiversity in the gut can lead to an increase in the prevalence of highly-virulent, fluoroquinolone-resistant *Clostridium difficile*, causing potentially deadly diarrhoea [21]. Although *C. difficile*-associated disease is rare in CF patients, ~50% of patients are colonised with the species and in many cases fatal *C. difficile* colitis in CF patients are reported in the literature [22,23].

There are also concerns regarding horizontal gene transfer (HGT) of resistance mutations between AMR-resistant strains and previously susceptible bacteria. Although HGT of AMR-genes has been demonstrated to be somewhat limited in an example study of the *E. coli* fecal flora following oral ciprofloxacin use [24], it seems certain that oral fluoroquinolone use does lead to an increase in the prevalence of AMR-organisms in the intestinal flora, particularly in the weeks following treatment [25]. Minimising drug exposure to the GI tract through using the inhaled route may therefore be beneficial in reducing the proliferation of AMR organisms in the gut following antimicrobial therapy.

3. Challenges for inhaled drug delivery

The rationale for the inhaled delivery of antibiotics is heavily dependent upon the ability to deliver high doses directly to the site of infection. Whilst inhalation should do this more efficiently than systemic therapies, there are still several challenges in being able to successfully deliver and sustain high antibiotic levels in the lung.

Achieving high concentrations of the drug in the airways depends upon the formulation being able to reach its target site in the lung following inhalation. When a nebulised droplet or a dry powder particle is inhaled, it is likely to deposit wherever it first makes contact with the airway surface [26]. The location of deposition is largely size dependent, with larger particles (>10 μm)

likely to deposit in the oropharyngeal region, intermediate particles (5 – 10 μm) depositing in the conducting airways (tracheobronchial region) and small particles reaching the alveoli (1-5 μm).

The region of deposition required for effective treatment will not be the same for all respiratory infections. In cases of pneumonia, infections tend to be found within the tracheobronchial region, whereas CF-associate *PA* infection is found primarily within the conducting airways [27]. Thus treating these two infections with an inhaled formulation would have two different ideal patterns of deposition i.e. evenly throughout the lung vs targeted to conducting airways for pneumonia and CF infection, respectively.

In the CF lung, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene result in the increased absorption of Na^+ and H_2O from the lung epithelium, changing the physical properties of mucus and reducing the effectiveness of mucociliary clearance [28]. Impaired mucus clearance leads to the formation of mucus plugs and plaques which can then become the site of bacterial infection. These plugs may be the desired targets for antimicrobial delivery, but by blocking airways they can also provide a barrier to prevent antibiotics from reaching the lower airways and infected foci there [27].

The infected lung is also a chemically harsh and challenging environment, and a drug must be able to withstand high salt concentrations, changes in oxygen levels, inactivating interactions with mucus and the need to penetrate bacterial biofilms in order to reach the bacterial organism and elicit its effect [29].

Finally, as the main purpose of inhaled antimicrobial delivery is to achieve high concentrations in the lung with minimal systemic absorption, it is also important that any drug administered remains at high levels (>MPC) within the lung lumen for a clinically useful time window following inhalation. This is important for chemical classes such as aminoglycosides and fluoroquinolones, which have concentration-dependent activity, and also for those classes such as beta-lactams for which activity is time-dependent, as it will increase the time at which the drug concentration is >MPC [10]. Molecular size, logP/lipophilicity and the existence of receptor-mediated transport mechanisms will all contribute to the rate of clearance of an antimicrobial from the lung following inhalation [30] and thus the extent to which it provides high and sustained drug concentrations to treat respiratory infection.

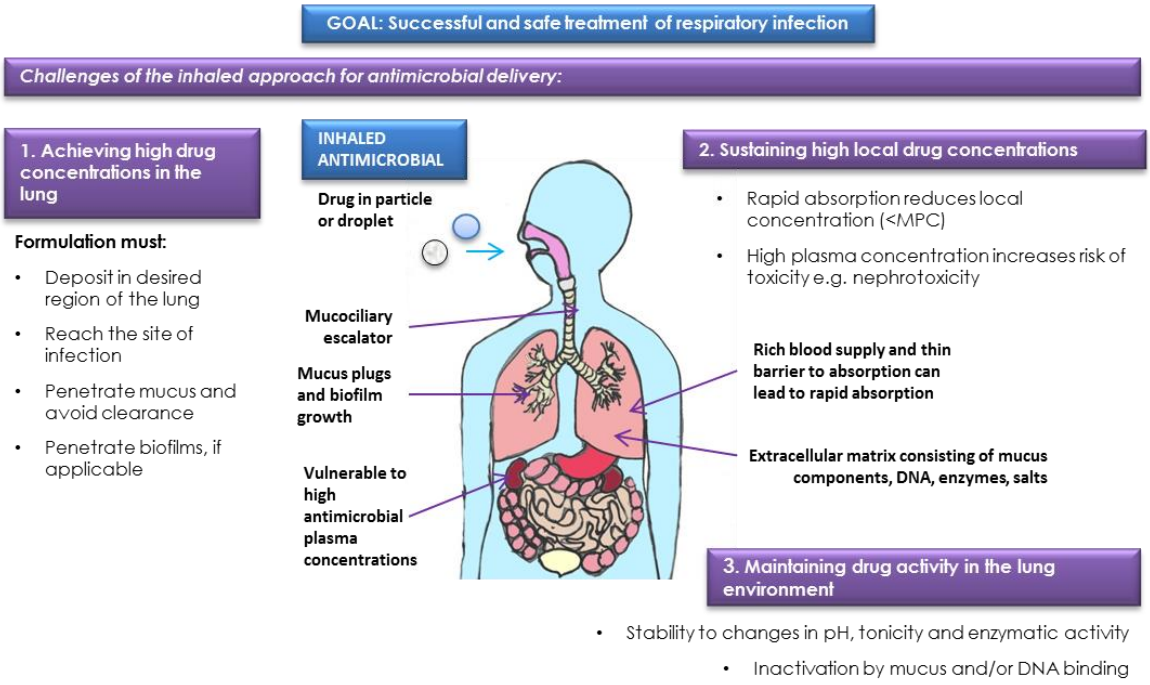
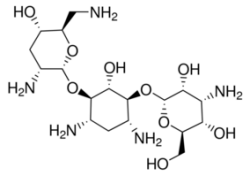
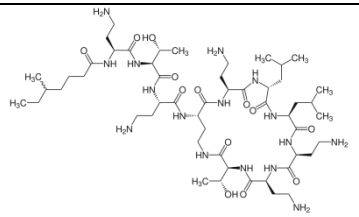
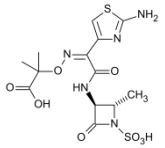
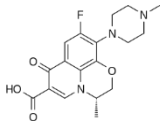


Figure 2. Challenges of inhaled drug delivery as an approach for respiratory infection treatment

4. Currently available chemical classes and development of products

There are a number of inhaled antibiotic formulations on the market which cover a several different chemical classes. In Europe, inhaled formulations of tobramycin (TOBI®, TOBI® Podhaler), aztreonam (Cayston®), colistin (Colobreathe®) and levofloxacin (Quinsair®) have been approved for use in the treatment of CF lung infection. Eight formulations of tobramycin (inc TOBI®, Bethkis®) and one formulation of aztreonam (Cayston®) have been approved by the FDA, and also for the inhaled treatment of CF infections. However, a wide breadth of research has been reported, showing the development of additional classes for inhalation, reporting sophisticated formulation strategies and the application of inhaled antimicrobials to other indications beyond CF *P. aeruginosa* infection. In the following section, the major classes of antibiotic which have been investigated for inhaled application will be discussed in terms of their molecular properties, their efficacy as an inhaled therapy and also the development of the drug into an effective formulation for pulmonary delivery, especially considering the particular benefits and challenges of each chemical class in these three areas.

Table 1. Current antimicrobial classes licensed for use as inhaled products in the EU and USA (accessed May 2017)

Chemical class	Drug	Structure	Products on market	Company	Formulation	References
Aminoglycosides	Tobramycin		TOBI® Podhaler (EU and USA)	Novartis Europharm Ltd	Dry powder	[31] [32] [33]
			Vantobra (EU)	PARI Pharma Gmbh	Solution	[34] [35]
			Bethkis® (USA)	Chiesi USA Inc	Solution	[36] [37]
			Kitabis Pak® (USA)	Pulmoflow Inc	Solution	
Polymyxins	Colistin		Colobreathe® (EU)	Teva B.V.	Dry powder	[38] [39]
Beta-lactams	Aztreonam		Cayston® (EU and USA)	Gilead Sciences International Limited	Solution	[40] [41] [42]
Fluoroquinolones	Levofloxacin		Quinsair® (EU)	Horizon Pharma Europe BV	Solution	[43] [44]

4.1 Aminoglycosides

4.1.1 Tobramycin

Chemistry and Molecular properties

Tobramycin, like other aminoglycosides, exhibits its antimicrobial mechanism of action by disrupting protein synthesis [45], and by the disruption of the outer cell membrane of gram negative bacteria [46]. Tobramycin's activity against gram negative bacteria, including *P. aeruginosa*, has made it a first-line treatment for chronic infection in the CF lung [47]. The oxygen and nitrogen-rich structure has a high aqueous solubility and a logP of -7.32 [48]. These physicochemical properties impart poor oral bioavailability [49], and thus systemic dosing must take place via intravenous administration. There is a great benefit to providing the drug in an alternative administration route.

The high solubility and low logP of tobramycin suggest it should have a long absorption half-life across the lung epithelium [30], facilitating high local concentrations of tobramycin. This has been demonstrated experimentally in an *in vitro* model of the human bronchial epithelium [48]. Tobramycin demonstrated limited permeability compared to other antibiotics studied, including *rifampicin* and *moxifloxacin*. Whilst rifampicin has a much larger MWt (822.94) than tobramycin, it has a significantly more positive logP (3.72). The reduced lipophilicity limits the permeability of the drug across the Calu-3 cell layer, and across the lung epithelium. This property is also attractive to reduce plasma tobramycin levels and avoid the nephrotoxic and ototoxic side effects associated with its use [15].

Tobramycin is relatively stable in aqueous solution and serum [50]. However, in CF mucus, tobramycin can bind to components such as mucins and DNA/DNA fragments, leading to the inactivation of the compound's antimicrobial activity [51]. The cationic charges of tobramycin at neutral pH give rise to electrostatic binding interactions with DNA, which then inactivate tobramycin [52]. Thus even higher concentrations of tobramycin can be required to achieve above-MIC levels of active tobramycin in the CF lung, to account for the inactivated fraction.

Efficacy of inhaled tobramycin

A number of large, multi-centre trials have demonstrated the efficacy of inhaled tobramycin in CF patients. Ramsey *et al* [53] demonstrated that inhaled tobramycin solution (600 mg daily, 28 day dosing period) resulted in significant improvements in lung function. Tobramycin administration was also associated with significant reductions ($P<0.001$) in *Pseudomonas aeruginosa* sputum density (~100 x) and a reduction in inflammatory markers (peripheral white blood cell counts and polymorphonuclear neutrophil counts). The inhalation of tobramycin was not associated with nephrotoxicity or ototoxicity. The success of inhaled tobramycin in improving lung function and reducing PA colonisation, coupled with the reduced cost compared to IV administration, identified this administration route as an attractive means to deliver effective antimicrobial chemotherapy to the infected lung.

PK and absorption of inhaled tobramycin

The pharmacokinetics of tobramycin following inhalation is of particular interest, due to the concerns regarding the safety of systemic aminoglycoside exposure. A safety and pharmacokinetic study conducted by Geller et al [54] in CF patients found that 95% of patients achieved sputum concentrations of >25 times the MIC, and 97.55 achieved > 10 times the MIC. Serum concentrations were significantly lower, with mean value of ~1 µg/mL. The median serum to sputum concentration ratio was 0.01, demonstrating high concentrations at the site of infection and limited systemic exposure.

The PK profile of tobramycin following does not appear to be significantly affected by the delivery device. Tobramycin solution administered twice daily for 2 weeks by either eFlow *rapid* (PARI GmBH, Stamberg, Germany) or PARI LC® PLUS (PARI) demonstrated comparable profiles following inhalation [55]. Sputum concentrations were variable between subjects (e.g. C_{max} = 981±1191 µg/gfor eFlow and 754±927 µg/g LC PLUS at day 1), but comparably high across the two devices.

The persistence of tobramycin in the airways is also of clinical interest and significance. Hubert *et al* [55] showed that following 15 days treatment, tobramycin levels were quantified *in sputum* before administration (148±354 µg/g and 65±107 µg/g for eFlow rapid and LC PLUS, respectively), suggesting long term accumulation of the drug upon repeat dosing. In contrast, Ruddy *et al* [56] found no significant difference between sputum levels at 1-2 days post 1st treatment and 24-28 days after 1st treatment. In both studies, sputum trough levels showed high levels of variation, meaning it was not possible to establish statistical significance between measurements. However, serum levels of tobramycin were extremely low at the end point of both studies, clearly confirming limited accumulation within the systemic circulation.

Formulation development

Early studies of tobramycin for inhalation employed IV tobramycin preparations, often containing antioxidants and preservatives, reconstituted with saline for nebulisation. A comparison of such a prepared solution with a preservative-free tobramycin solution in CF patients indicated that although both solutions were associated with bronchospasm, a reduction was observed for low-risk patients when using the preservative-free preparation [57]. Importantly, the preservative-free formulation contained a 3-fold higher dose of tobramycin than the IV solution but was better tolerated, confirming that the preservatives were responsible for the bronchospasm response, not tobramycin itself.

Tobramycin solution for inhalation (TOBI®) was developed specifically for inhalation purposes. The solution pH and osmolality were developed to match that of the lung, and the formulation was prepared without phenol or sodium metabisulphite preservatives, which have been previously associated with bronchospasm [58]. Ramsay *et al* [53] demonstrated the tolerability of TOBI® in the treatment of CF-associated PA infection, with no observed increase in bronchospasm following the administration, compared to a placebo.

Bramitob®, developed by Chiesi, is a second approved formulation for the administration of tobramycin to the lung. Bramitob® comprises of 300 mg tobramycin in 4 mL of preservative-free 0.45% saline solution. The increased saline concentration increases the osmolality of the solution, close to that of fluid in the CF lung [59]. The decreased saline volume, and consequent decrease in nebulisation time, was designed to reduce treatment burden and therefore improve patient compliance [36]. A comparison of *in vitro* aerosol performance and *in vivo*

pharmacokinetics of Bramitob® vs TOBI® demonstrated no significant difference between the formulations, with the exception of slightly higher drug levels *in sputum* at 30 mins post-inhalation, a likely consequence of the 25% higher drug concentration within the formulation [60]. Recently, an extensive two-phase study comparing the long term (56 weeks) efficacy and safety of Bramitob® and TOBI® again concluded that clinical outcomes were comparable between the two formulations [37]. Both formulations have been demonstrated to be comparable in terms of their *in vitro* antimicrobial efficacy against clinical *PA* CF isolates [61].

Recently, Greise *et al* [34] have reported the use of a highly concentrated, low volume tobramycin solution for inhalation (150 mg/ 1.5 mL, T100 PARI), designed to further reduce lengthy nebulisation routines and improve patient compliance. The inhalation of T100 PARI was associated with significantly reduced plasma concentrations compared to TOBI®, leading to an improvement in the plasma/sputum ratio (%) as well the reduction in nebulisation time (4.6 mins for T100 pARI vs 16.1 mins for TOBI®). Similar levels of adverse events were associated with both solutions; however, a higher number of adverse events associated with the "ear and labyrinth" system were reported for the T100 study. The majority of these events were assigned as unrelated to the treatment - however, further investigation of this observation with a larger study cohort should elucidate the significance of this finding.

Formulation of tobramycin – Development of dry powder formulation

Tobramycin solution for inhalation has been demonstrated to be effective - however, administration times for nebulised tobramycin can be lengthy (~15 – 22 mins [37]), which affects patient compliance with therapy. The time spent setting up nebulisers, cleaning equipment and administering the therapy contributes to even longer times in total for nebulised therapy (~41 mins per day for adult CF patients [62]). Dry powder formulations enable the administration of inhaled antibiotics from a simple, convenient handheld device. The ease of use and reduction in treatment burden is popular with patients [63] and can have a significant benefit on their quality of life.

PulmoSphere® technology has been employed to prepare high performance dry powder formulations of tobramycin for inhalation. PulmoSphere®'s bottom-up particle engineering allows the preparation of uniform hollow, porous spherical particles with reduced density, which offers improved aerosolisation properties compared to conventional micronized products [64]. Tobramycin PulmoSphere® particles are formulated with Distearoylphosphatidylcholine (DSPC) as the surfactant, which helps to stabilise the droplets formed in the spray-drying process. During droplet drying, the DSPC aligns at the particle surface, improving the powder flowability and facilitating the dispersion of the dry powder from an inhaler device with minimal input of energy [31]. This allows the dry powder to be effective when the inhaler device is actuated by patients with impaired lung function, as is expected from CF patients. The dry powder is loaded into capsules to protect the amorphous solid from humidity, and is loaded into a handheld inhaler device for administration.

A comparative phase 1 study of the PK and safety of tobramycin inhalation powder (TIP) vs TIS in CF patients demonstrated similar drug levels in the serum following either treatment (300 mg TIS vs 112 mg TIP) [65]. A dose-dependent trend towards increased sputum levels following powder administration was observed, although this was not significant due to high levels of variation. The most marked difference between the two formulations was in administration time, with a

reduction from 15.8 min for inhalation of 300 mg TSI vs 4.9 min for 112 mg of TIP, indicating the benefit of the powder formulation in terms of convenience for the patient. In terms of efficacy, TIP has been demonstrated to be non-inferior to TOBI®, with respect to preventing loss of lung function (FEV_1) and reduction in bacterial sputum density [32]. TIP has been demonstrated to be well tolerated following long term administration (1 year), with the most commonly reported adverse event associated with treatment to be a cough [33]. This one-year, open-label phase IV trial also noted the sustained suppression of *PA* over the 12 month study period, demonstrating long term antimicrobial efficacy as well as safety. Increased adherence and decreased requirement for intravenous antibiotics following the use of TIP has been demonstrated in a 'real-world' study of TIP use in the clinic, showing that these benefits are also observed by patients and clinicians outside the controlled structure of a clinical trial [66].

TIP was initially developed for delivery using the T-326 Inhaler, later marketed as the Podhaler® (Novartis Pharmaceuticals, California, USA). The capsule-based device has a low airflow resistance, which facilitates the generation of rapid airflow by the patient to aid in powder dispersion and deposition [31]. The development of high-dose disposable devices has recently been reported; they enable the use of pure drug powders for the inhalation of aminoglycosides. The Cyclops [67] and Orbital [68] devices are both single-use, capsule-free devices that have been recently reported to improve thee dry powder delivery of tobramycin and improve convenience for the patient. Pre-clinical and clinical assessments of these devices and comparison to current technologies will be of great interest to facilitate innovation in tobramycin delivery to the lung.

4.1.2 Amikacin

Molecular properties

Like tobramycin, amikacin is a polycationic aminoglycoside that is highly soluble in aqueous media. The molecule is a weak base, which has multiple cationic sites at pH 7 [69]. These molecular properties result in poor oral bioavailability for amikacin, due to its limited uptake across the gut epithelium [70]. The poor absorption profile of amikacin is, however, of benefit in inhaled delivery. The charged structure of amikacin at the lung pH (~6.6 -7.1 in the healthy lung, lower during infection [71]) would suggest limited absorption across the lung epithelium, and thus the ability to achieve high local concentrations of amikacin in the airway following inhalation.

Amikacin is of great interest as an inhaled therapeutic, as it has maintained potent antimicrobial activity against clinical MDR- respiratory pathogens including *PA* and *Klebsiella pneuoniae* [72]. In a panel of 2460 blood and respiratory isolates collected from across 50 US hospitals, 96% of *E. coli* and *K. pneumoniae* isolates had an amikacin MIC <16 mg/mL and 95% of *PA* isolates had amikacin MICs of <16 mg/mL, showing the clear rationale behind administering this drug to the infected lung.

Efficacy of inhaled amikacin

The efficacy of inhaled amikacin against *PA* infection in the CF lung was demonstrated in an early study by Schaad *et al* [73], comparing inhaled amikacin administered in combination with IV combination ceftazidime (250 mg/kg/day) + amikacin (33 mg/kg/day), or the IV treatment alone. Patients treated with inhaled amikacin demonstrated significantly greater success of *PA*

eradication *in sputum* (P<0.02), and this response was demonstrated to be significantly linked to the amikacin concentration achieved in the lung.

AS well as CF-associated *P. aeruginosa* infection, amikacin has also been employed as an inhaled treatment for a variety of other pulmonary infections. Goldstein and colleagues [13] compared the lung targeting and efficacy of inhaled vs IV amikacin in a model of ventilator-acquired *Eschicheria coli* pneumonia in piglets. Lung concentrations of amikacin were 3-30 fold higher in the inhaled treatment group compared to control (P<0.01). Lung tissue samples from the aerosol-treated group were found to have lower bacterial burden than those from the IV treated group, and a significantly greater number were found to be free of *E. coli*. Inhaled amikacin has also been reported in the successful treatment of other challenging clinical species, including multidrug-resistant *Staphylococcus aureus[74]* and *Mycobacterium abscessus* [75], indicating the versatility of this treatment for respiratory infection.

PK of inhaled amikacin

The high water solubility and similar molecular size of amikacin and tobramycin infer that these two molecules would demonstrate similar pharmacokinetic profiles following inhalation [76]. This has been demonstrated widely throughout the literature. Ehrmann and colleagues [77] compared the pharmacokinetics of intravenous and high-dose nebulised amikacin administered under mechanical ventilation and found the median peak serum concentration was ~4-5 fold higher with IV dosing compared to inhalation, despite the significantly lower administered dose (15 mg/kg vs 40-60 mg/kg).

A PK study of BAY41-6551, a drug-device combination comprising of amikacin formulated for inhalation and an aerosol delivery platform, in mechanically-ventilated nosocomial pneumonia patients again illustrated significantly lower serum levels than lung concentrations [78]. Repeat administration of amikacin to the lung through inhalation has not been demonstrated to result in accumulation in lung sputum or plasma in *in vivo* models [79] and in clinical settings [80], with the exception of patients with severe renal impairment who require dialysis [81].

Formulation of inhaled amikacin

The high aqueous solubility of amikacin enables the preparation of highly concentrated solutions for inhalation, a significant benefit in achieving maximum ELF/sputum lung concentrations and reducing administration time. Amikacin Inhale is a specially formulated solution-device combination for aerosol administration of amikacin. It contains 125 mg/mL amikacin sulphate in solution, which is designed in terms of osmolality for use in the human lung [78]. It is preservative-free, allaying concerns regarding preservative-associated toxicity in the airways. Delivered in conjunction with the Pulmonary Drug Delivery System, or PDDS, (Nektar Therapeutics, San Francisco, USA) vibrating mesh nebuliser the formulation was determined to offer a good pharmacokinetic profile in mechanically ventilated pneumonia patients, with high ELF amikacin concentrations and minimal systemic absorption, as determined from serum drug levels.

The addition of surfactant to amikacin solution has been proposed as a means to improve the aerosol deposition in the lung upon inhalation [82]. Amikacin delivered in combination with sterile exogenous porcine surfactant (Suzacrin, Docpharm, Ukraine) was demonstrated to have improved the antimicrobial efficacy compared to amikacin alone. This effect was attributed to the more efficient delivery of amikacin possible from a surfactant-containing solution, and would be

an interesting area for further investigation as an improvement to solution-based amikacin therapies.

Formulation of amikacin for controlled release: Liposomal amikacin

A major challenge in respiratory infection treatment is in delivering effective therapy for biofilm infection. The thick, oxygen-poor mucus plugs of the CF lung create anaerobic environments, which induce the formation of *Pseudomonas aeruginosa* biofilms [83]. The mucus binding properties of aminoglycosides can reduce the ability of these drugs to penetrate biofilms, requiring a formulation intervention to successfully reach organisms and exert an antimicrobial effect.

Liposomes containing amikacin have been demonstrated to be capable of permeating *PA* biofilms in *in vitro* studies, leading to an increase in antimicrobial activity of liposomal amikacin compared to free drug molecules (amikacin or tobramycin) in the pre-clinical model of infection [84]. Once-daily liposomal amikacin was demonstrated to be as effective against mucoid *PA* infection (in a rat model) compared to twice-daily free tobramycin. Liposomal amikacin demonstrated longer lung retention compared to free tobramycin; this difference in lung PK enables a sustained high drug concentration at the infection site for hours following inhalation. Additional contributing factors to the efficacy of liposomal amikacin include facilitating the improved uptake of the drug through the bacterial membrane and protection from degradation/inactivation of the drug molecule in CF sputum [85] .

In clinical studies, liposomal amikacin was well tolerated by CF patients and did not induce renal or audiological damage [86]. In a Phase II study, liposomal amikacin administered 280 and 560 mg liposomal amikacin (Arikace®) daily demonstrated sustained and significant improvements in FEV_1 and reductions in *PA* sputum density in these two treatment groups relative to placebo group. A Phase 3 trial of Arikace® has been completed (NCT01315678), although to the best of the authors' knowledge, no results have been published. The progress of Arikace® will be of great interest in the development of further novel formulation approaches for inhaled antibiotics.

As well as CF-associated *Pa* infection, liposomal amikacin has also been investigated in pre-clinical trials against non-tuberculosis mycobacterium respiratory infection. Liposomal amikacin was demonstrated to be able to penetrate into *Mycobacterium avium* subsp. *hominissuis* (*MAH*) and *Mycobacterium abscessus* (*Ma*)-infected macrophages, significantly improving the *in vitro* antimicrobial efficacy compared to free amikacin [87]. This translated to efficacy *in vivo* in a mouse model of MAH infection when liposomal amikacin demonstrated comparable, although not significantly superior, efficacy compared to parenteral free amikacin administration. Further investigations to compare the efficacy of inhaled free vs inhaled liposomal amikacin against intracellular pathogens would be of significant interest.

4.2 Polymyxins

4.2.1 Colistin

Molecular properties

Colistin is a polypeptide antibiotic from the polymyxin family isolated from the soil bacterium *Bacillus colistinus [88]*. It has increased in importance in recent years as one of the few remaining therapeutic options for multi-drug resistant gram negative infections [89]. It is available in two chemical forms: colistin sulphate and colistimethate sodium (CMS), both of which can be administered via inhalation. Colistimethate sodium is a pro-drug, hydrolysed *in vivo* to the active form of the drug [90]. Colistin has been associated with neurotoxicity and nephrotoxicity, the precise mechanisms of which are unknown but may be linked to its ability to permeabilise epithelial membranes [91]. Colistin, delivered as colistimethate sodium, has been demonstrated to have improved activity against *P. aeruginosa* biofilms *in vitro* under "CF-like" conditions, using anaerobic conditions and a slightly acidic pH of 6.4 [92] demonstrating that it should retain its activity even in the challenging physiological environment of the infected lung.

As a polypeptide, colistin is relatively large in molecular weight and contains many hydrophilic functional groups. However, it also possesses a lipophillic fatty acid tail which is vital to its antimicrobial activity. The tail is believed to disrupt the outer membrane of gram negative bacteria, increasing drug uptake into the cell [93]. Colistin sulphate has a molecular weight of 1,155.4 and logP of -2.4 [94], both indicating a propensity for slow absorption half-life following inhalation[30]. Colistin demonstrates poor penetration from the bloodstream into the pulmonary lining fluid compared to other antimicrobials, including minocycline and gentamycin [95]. Local administration directly to the lung is therefore attractive to increase the achievable airway concentrations and to reduce systemic exposure and associated nephrotoxicity concerns.

Efficacy of inhaled colistin

Inhaled colistin therapy has been demonstrated to be highly effective in a number of pre-clinical and clinical studies. In a pre-clinical porcine model of *Pseudomonas aeruginosa* ventilator-associated pneumonia, inhaled colistin was demonstrated to be significantly more effective than intravenous therapy in terms of lung targeting and reduction of bacterial lung burden [96]. An early trial of inhaled CMS solution in *P aeruginosa*-infected CF patients demonstrated a small reduction in loss of lung function over treatment period (90 days) however no eradication of *P. aeruginosa* was observed. [97]. Studies employing inhaled colistin as an additional therapy illustrated significant improvements in treatment outcomes. Frederiksen *et al* [98] demonstrated improvemenst in lung function, *Pa* eradication and reduced hospitalisation following early intervention in CF patients with combination inhaled colistin/oral ciprofloxacin treatment. Adjunctive aerosolised colistin therapy was also reported for the treatment of MDR-*P. aeruginosa* caused-nocosomial pneumonia and tracheobronchitis [99].

Given concerns regarding its toxicity, the comparison between colistin and other inhaled antibiotics is of interest. Colistin has been demonstrated to be somewhat less beneficial in the treatment of CF-associated *P. aeruginosa* compared to inhaled tobramycin. In comparable treatment groups, administration of TOBI® was observed to result in both a significant reduction

in *P. aeruginosa* and an improvement in lung function, whereas colistin therapy was only observed to reduce bacterial density [100]. It is important to note that adverse effects have been reported following inhalation of colistin therapy including bronchosconstriction [101], chest tightness [102], airway hypersensitivity [103] and hypotension [104]. Although these serious adverse effects are rare, they are indicative of the potential toxicity of colistin largely believed to be due to its physicochemical properties e.g. its ability to be membrane active.

However, colistin still remains an attractive treatment option due to its capacity to treat multidrug-resistant infections. MDR-*Acinetobacter baumanii* has been identified by the CDC as serious public health threat requiring urgent action [21]. Ventilator-associated and community-acquired pneumonia have both been associated with *A. baumanii* infection [105]. In a retrospective matched case-control study, the treatment of MDR- *A. baumanii* infection with inhaled CMS (160 mg) was evaluated in patients ≥18 years old [106]. Treatment with inhaled CMS resulted in significantly faster eradication of MDR-*A. baumanii* than in the control group. Treatment with inhaled CMS was increased cost of treatment by USD$ 115±38, however this was small in magnitude when compared to the additional cost of prolonged isolation in the control group (~USD$ 1600-2400). No significant difference in bronchospasm or nephrotoxicity was observed, suggesting the treatment was also well tolerated. Aerosolized colistin has also been reported as a highly successful monotherapy for *A. baumanii* pneumonia in pre-term and non-premature infants, where inhaled colistin can provide an extremely important alternative to IV therapy when intravenous catheters cannot be successfully placed, and patients could otherwise not be treated [107].

PK of colistin

Pre-clinical and clinical studies of inhaled colistin have demonstrated the targeting ability of this mode of delivery to achieve high concentrations of drug in the lung compared to intravenous delivery. Lu et al [96] observed median peak lung tissue concentrations in piglets following inhalation of 8 mg/kg CMS to equal to 2.8μg/g tissue, but found colistin to be undetectable in any lung tissue sample collected following intravenous administration. The pharmacokinetics of inhaled vs IV colistin has been investigated in a clinical study in 12 adult VAP patients by Boisson et al [108]. Colistin levels were demonstrated to be far higher in ELF and far lower in plasma following the aerosol delivery compared to following IV administration (ELF levels ~9.5 – 1,137 mg/L inhaled delivery vs ~1.5-28.9 mg/L IV dosing). Repeated IV administration did not result in accumulation of colistin in either ELF of plasma.

Following the demonstrated success of aerosolised colistin treatment in neonates with VAP, Nakwan and colleagues [109] reported the PK profiles following inhaled delivery of colistin (as CMS, 4 mg/kg colistin base activity) to six neonatal patients with either *AB* or *Klebsiella pneumoniae*-caused VAP. As in agreement with previous studies, plasma levels were markedly lower (mean C_{max} ~0.6 μg/mL) than tracheal aspirates demonstrating a targeted delivery to the airways, even in the neonatal lung under ventilation.

Formulation of colistin for inhalation

The vast majority of *in vitro*, pre-clinical and clinical studies with inhaled colistin reported in the literature employ CMS reconstituted in saline or sterile water.

A blinded clinical study comparing the tolerability of the same equivalent dose of colistin (67 mg/6 mL) delivered as either colistin sulphate or colistin sulphomethate (both in 0.9% saline) illustrated clear differences between the two formulations [110]. Of the 9 study participants, 7

were unable to complete colistin sulphate nebulisation due to throat irritation and severe cough. Although CMS administration did result in a reduction in FEV_1 at 15 min post treatment for 2 patients, no other effects were reported demonstrating the improved tolerability of the pro-drug.

Unlike tobramycin or amikacin, CMS delivers colistin in a prodrug form. Any administration and dosage calculation must therefore include a calculation of the bioavailability of colistin following conversion from the inactive form. In *in vitro* stability studies, CMS has been observed to hydrolyse to form colistin following incubation in water, PBS and human serum. The maximum concentration of colistin was reached following ~12-24 h incubation in serum at 37 °C, followed by a decrease attributed to the instability of colistin in serum [111] . CMS levels are significantly higher (~4-20 fold) than active colistin levels in the ELF [108] and so high dose formulations able to achieve therapeutic active drug concentrations are vital with CMS inhaled delivery.

Formulation of colistin for inhalation – Formulation developments

Major innovations in the formulation of colistin pulmonary use have focused on reducing the burden of treatment on the patient by developing a dry powder formulation.

An early study of the tolerability and PK of a colistin dry powder formulated for inhalation using an ordered mixture of colistin sulfate and lactose demonstrated poor tolerability in CF patients, causing moderate cough and reductions in lung capacity [112].. Further development of a dry powder colistin formulation has employed the CMS prodrug, rather than active colistin sulphate. Westerman and colleagues [113] reported that a dry powder formulation of CMS (CMS 83.3%, lactose sweeper crystals 16.7%, total dose 25 mg CMS and 5mg lactose) was better tolerated by CF patients than the previously reported colistin formulation, with no clinically relevant reduction in FEV_1 up to 30 mins post inhalation.

The safety, efficacy and patient acceptance of a colistin sulphomethate dry powder formulation, Colobreathe®, delivered in the Turbospin (PH&T, Milan, Italy) dry powder inhaler (DPI) was investigated in a phase 3, open label study in CF patients ≥ 6 years of age [38]. Colobreathe DPI was demonstrated to be non-inferior to TOBI® (Novartis Pharmaceuticals, Basel, Switzerland) in terms of the change in FEV_1 over the study period. The DPI was well tolerated by the participants, although adverse events of cough and abnormal taste were reported more frequently than for the nebulised solution, as can be a disadvantage for dry powder-based therapies. Administration of Colobreathe® did not result in a change in the susceptibility of *Pa* to colistin during the study period, which is of considerable importance given the 'last chance' status of colistin as an antimicrobial. Significantly more patients reported the Colobreathe as being easy or very easy to use than the TIS/Pari LC nebuliser treatment, and of those patients who had previously used both treatments, a greater proportion 65.6% of Intention to Treat (ITT) patients preferred the dry powder treatment. Colobreathe® was approved by the EMA in 2012. Further development of colistin dry powders has been reported in terms of exploring alternative powder formulations, for example the use of spray-dried CMS with potentially improved aerosolisation properties than the current milled powders [114]. Given the impressive aerosolisation properties obtained through particle engineering of tobramycin using PulmoSphere technology, this looks to be an interesting area of future inhaled product development.

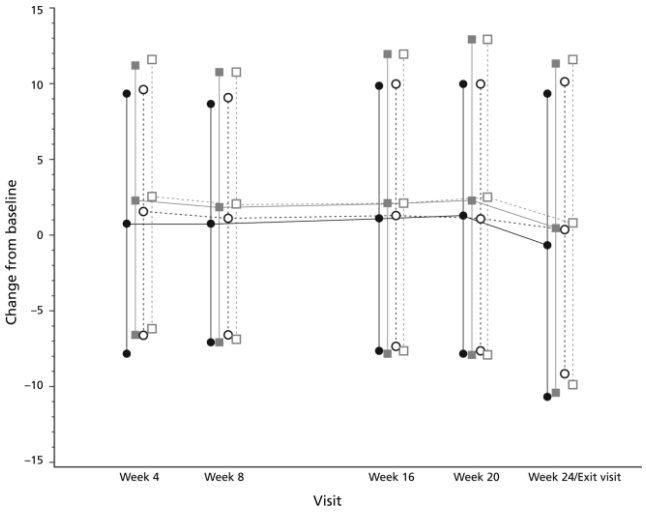


Figure 3. Comparison of changes in FEV_1 % predicted from baseline to week 24 following treatment with Colobreathe® and tobramycin solution for inhalation (TIS) (black circles - intention to treat population (ITT) Colobreathe®, grey squares – ITT TIS, open circles – completed Colobreathe®, open squares – completed TIS

Table 2. Patient ease of use of Colobreathe® dry powder vs TIS nebulised solution showing significant (P<0.001) improvement in patient response to the dry powder formulation. Figure and table taken from Schuster et al (2014)

Patient response, n (%)	Colobreathe® Dry Powder (n=183)	Tobramycin solution for inhalation (n=191)	Overall (n=374)	95% C.I.	P value
Very easy to use	95 (51.9)	19 (9.9)	114 (30.5)	4.684, 15.274	<0.001
Easy to use	71.8 (38.8)	84 (44.0)	155 (41.4)		
Neither easy nor hard to use	4 (2.2)	61 (31.9)	65 (17.4)		
Hard to use	6 (3.3)	16 (8.4)	22 (5.9)		
Very hard to use	1 (0.5)	3 (1.6)	4 (1.1)		
Missing	6 (3.3)	8 (4.2)	14 (3.7)		

4.3 Beta lactams

4.3.1 Aztreonam

Molecular properties

Aztreonam is a monobactam which demonstrates good activity against a range of gram negative aerobic bacteria, including *Eschicheria coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [115]. The molecule has a molecular weight of 435.4 [116] and is stable at 37°C in aqueous solution for at least 24 h [117]. Empirical and computational studies have suggested that the carboxylic acid and sulfonyl groups are deprotonated at physiological pH, giving the molecule dianionic charges [118].

Aztreonam demonstrates very poor oral bioavailability. Less than 1% of the administered dose (500 mg) was quantified in serum following oral dosing; this limited absorption has been attributed to the polar moieties of the chemical structure impeding transport through the lipid bilayer [119].Poor absorption across the intestinal epithelium also results in difficulty crossing from the bloodstream to the lung tissue. Sputum samples collected following intramuscular administration of 1 – 2 g of aztreonam have indicated that ~3.5-4.5% of the total dose penetrated into the lung lining fluid from the systemic circulation [120]. For this reason, aztreonam must be administered by intramuscular injection [115] and there is a clear clinical benefit in developing an inhaled formulation for less invasive delivery and to achieve higher local lung concentrations with lower systemic exposure.

Efficacy of inhaled aztreonam

Inhaled aztreonam demonstrated impressive efficacy in *in vitro* and clinical PK assays [40]. The inhaled formulation demonstrated good activity *in vitro* against *P. aeruginosa* and did not demonstrate inactivation by CF sputum, in contrast to tobramycin which was investigated as a control. Doses of >225 mg aztreonam were well tolerated by CF patients, with few adverse events reported. The formulation demonstrated high local lung drug concentrations (medium sputum concentration 985 µg/g) and low serum levels (~400 ng/mL).

A Phase II, double blind, placebo-controlled trial in CF patients was performed to investigate the PD efficacy of the aztreonam lysinate formulation for inhalation (AZLI) [41]. AZLI resulted in significantly decreased bacterial density relative to placebo controls, with a significant dose-dependent effect at days 7 and 14 ($P<0.001$). Lung function data did not illustrate such a dose-dependent effect. FEV_1 increased at 7 days following 75 and 225 mg dosing, however for 225 mg mean change in FEV_1 dropped again closer to baseline by day 14. Overall, no significant effect on lung capacity was observed for AZLI. The formulation was well-tolerated, with no differences in AEs reported between the two treatment groups and control group. The most commonly reported AE was cough, which was considered to be treatment-related in 15 patients. For this symptom, a possible dose-dependent effect was observed with an increase in patients suffering mild cough as AZLI dose increased from 0 – 225 mg.

Long-term follow-up studies following patients using AZLI (75 mg, either two or three times daily) for up to 9 cycles of 28-days on AZLI/28 days off showed mean improvement in FEV_1 at the end of each treatment course, and a return to baseline levels after 1 month off treatment [12]. Treatment benefits were slightly greater following thrice-daily administration compared to twice daily. The total time that bacteria are exposed to aztreonam and other beta-lactams is for the efficacy of bacterial killing [116], which would support the findings of this study. A decrease in *P. aeruginosa* sputum density was observed during each of the treatment cycles, demonstrating that 75 mg thrice-daily AZLI was an effective suppressive therapy for *Pseudomonas* infection in

CF patients. The large, multi-centre, open label ALPINE study also demonstrated the tolerability and efficacy of aztreonam lysine treatment for recently detected *PA* infection in children [42].

A randomized, open-label clinical study comparing the efficacy of inhaled AZLI vs TOBI® therapy in CF patients showed greater improvement in lung function (FEV_1) following three cycles of 28 day on, 28day off AZLI treatment [121]. In this study, an optional extension period of 3 further AZLI treatment cycles was completed by patients from both study groups. Patients in the AZLI/AZLI (1st study/extension) group showed increases in body weight throughout the study period. For those in the TOBI/AZLI group, weight loss was observed during TOBI® treatment followed by weight gain during AZLI treatment, which was coupled with an improvement in FEV-1%. Both treatment groups demonstrated comparable numbers of patients with MDR-*PA* at the end of the treatment period, indicating no significant difference in the impact of each drug on the lung microflora.

Two multi-centre phase 3 studies investigating AZLI for the treatment of bronchiectasis (NCT01313624 and NCT01314716) have reported outcomes indicating that AZLI did not provide a significant clinical benefit compared to placebo for treatment of this condition [122]. Further investigation is required to identify the reason for this given the demonstrated success of the formulation for CF-associated *Pa* infection.

PK of aztreonam and role in inhalation

Aztreonam has been demonstrated to show very limited diffusion across the lung epithelium following inhalation in a number of studies. Gibson and colleagues [40] quantified high sputum drug levels following inhalation of aztreonam delivered as 175 mg aztreonam lysinate at 3 concentrations (175 mg/mL (1 mL), 75 mg/mL (2 mL) and 75 mg/mL (3 mL)). Sputum levels reached peak values at 10 mins, but remained ≥ the MIC_{50} for *Pa* (<2 µg/mL) for at least 4h. Mean plasma concentrations peaked at 419 ng/mL, illustrating limited drug absorption into the systemic circulation as would be predicted. Similar results were reported in phase-II clinical studies, with high sputum concentrations and limited permeation into serum [41]. In phase II studies, sputum drug concentrations were determined to be proportional to the dose administered. However as with previous reports of inhaled antibiotic administration, sputum levels were highly variable e.g. in the 225 mg AZLI treatment group, the overall range was 80.2 – 5680.0 µg/g. This demonstrates an important challenge with regards to inhaled delivery, as it is hard to predict the exact drug deposition following inhalation. Formulation approaches such as engineered dry powder technology has been used to address this issue, and will be discussed in detail in this review.

Formulation of aztreonam for inhalation

The first pre-clinical and phase 1b study investigating aztreonam for inhalation was reported by Gibson et al in 2006 [40]. The study employed a novel formulation of aztreonam, aztreonam lysinate, which had been designed specifically for inhalation in order to replace the aztreonam arginate salt employed in parenteral delivery. The arginate salt was replaced due to concerns regarding the role of arginine in nitric oxide production in the lung, and the possibility for this to induce increased airway reactivity and inflammation [123].

The AZLI formulation, marketed as Cayston® (Gilead Sciences, Foster City, CA), has been specifically developed in order to be suitable for administration to the lung using the Altera®

Nebuliser System (PARI), which delivers the 75 mg dose over a relatively short time window of 2-3 minutes [124]. AZLI contains lyophilised aztreonam lysine which is reconstituted with sterile 0.17% saline [116]. This formulation has been reported to have a relatively mono-dispersed particle distribution in a suitable size range for deposition in the lower respiratory tract (mass median diameter reported as 3.8 μM) and has been demonstrated to be well tolerated in a number of studies [41,42].

Formulation of aztreonam – future directions

AZLI demonstrates very good clinical efficacy. However, at present it is only available in solution form for delivery via a nebuliser. Nebuliser delivery requires lengthy set-up and cleaning procedures, which then can result in significant treatment burden for patient and carers [125]. Little has been reported on the development of aztreonam dry powder formulations in the literature. However, patents describing the preparation and efficacy of dry powder formulations of aztreonam lysinate for inhalation have been filed [126,127] and thus would suggest that products will emerge that fill this formulation design space.

4.4 Fluoroquinolones

4.4.1 Ciprofloxacin

Molecular properties

Ciprofloxacin is a fluoroquinolone derivative with mode of action via inhibition of bacterial DNA gyrase. Ciprofloxacin prevents the resealing of double-stranded DNA following the introduction of negative supercoils into the DNA helix by DNA gyrase, leaving the single stranded DNA liable to degradation [128].

The hydrochloride salt of ciprofloxacin is commonly used in formulation to improve aqueous solubility. It has an experimentally derived log D of -1.70 [129]. The aqueous solubility of ciprofloxacin is highly dependent on pH. At neutral pH it is sparingly soluble (0.15 ±0.006 mg/mL), whereas at low or high pH its solubility increases due to the changing ionisation state of the molecule [129]. It demonstrates good oral bioavailability, and is most commonly administered twice daily via the oral route [128]. The modest molecular weight and increased lipophilicity when in its uncharged state at physiological pH would be expected to result in an increased permeation across the respiratory epithelium following inhalation [30], and this has been demonstrated in cell culture studies. *In vitro* transport studies of ciprofloxacin transport across the Calu-3 bronchial epithelial cell culture model showed ~81% of 10 μM ciprofloxacin and ~49% of 20 μM ciprofloxacin was transported in the apical to basal direction over 180 min exposure [48]. The rate of transport was not dependent on initial drug concentration, suggesting that the transport of ciprofloxacin may involve the role of drug transporters as well as passive diffusion. Drug transporters are likely to play an important role in the fate of ciprofloxacin in the lung [130].

Efficacy of inhaled ciprofloxacin

The majority of published data regarding ciprofloxacin has studied advanced formulations of the drug for inhaled delivery (e.g. engineered dry powder and liposomal formulations). However, the efficacy of 'free' ciprofloxacin, delivered in a solution formulation, can be determined from these studies in which it is used as a control. In a pre-clinical evaluation of the efficacy of aerosolised free vs liposome encapsulated ciprofloxacin in a mouse model of *Francisella tularensis* infection, ciprofloxacin was determined to offer little or no protection against infection when delivered as an aerosolised solution [131]. All mice treated with unencapsulated ciprofloxacin succumbed to infection by day 9. Similar results were observed again by Wong and colleagues [132] in the same model of tularensis infection.

Chono and colleagues [133] investigated the potential efficacy of intratracheal-delivered ciprofloxacin by calculating the ratio of lung drug levels as quantified in a PK study in rats to the MICs of a suite of intracellular and extracellular pathogens. The C_{max}/MIC ratio was far higher following intrapulmonary delivery of ciprofloxacin solution compared to oral administration. For the intracellular pathogens, for *Mycobacterium tuberculosis* the calculated C_{max}/MIC ratio was 79 for pulmonary delivery compared to 12 following oral delivery. *P. aeruginosa*, an extracellular pathogen, showed the same significant targeting benefit for inhaled delivery with C_{max}/MIC ratios of 35 and 0 for pulmonary and oral delivery respectively. Although, importantly, bacterial killing efficacy was not demonstrated thus making these values theoretical indicators and not evidence of the true efficacy of the therapy.

Pharmacokinetics of inhaled ciprofloxacin

The relatively high permeability of un-encapsulated ciprofloxacin seen in cell culture models has been reflected in the limited PK data which has been reported for inhaled ciprofloxacin solution. In the study of Wong et al [132], immediately following administration of 2.2 mg ciprofloxacin to the mouse lung via nebulisation, drug levels in the lung were measured as ~16 µg/g tissue. The levels were comparable to those achieved using the same dose of liposomal formulation. However, dosing with free ciprofloxacin resulted in a rapid decrease in drug levels by 2 h post-administration, with an estimated clearance half-life estimated to be 1h. This was in stark contrast to liposomal lipofloxacin which was retained in the lung for up to 12 h.

In comparison with systemic administration, pulmonary delivery of ciprofloxacin has been demonstrated to achieve higher local lung concentrations. A pre-clinical study comparing the PK of ciprofloxacin delivered via intratracheal dosing (200 µg/kg) and through oral administration (10 mg/kg) [133] found clear increases in drug ELF concentrations following intrapulmonary delivery compared to oral. ELF C_{max} was determined to be 17.6 ±1.4µg/mL following IT vs 0.15 ±0.02 µg/mL following oral administration, despite the far higher dose used in oral delivery. Plasma levels were higher following oral delivery (C_{max} 0.35 ±0.03 µg/mL oral vs 0.14±0.04 µg/mL IT), however the difference was not as large as that observed for other inhaled antibiotics indicating increased uptake into the bloodstream. Alveolar macrophage levels were also greater following IT delivery (C_{max} 39.6 ±8.2 µg/mL vs 5.8 ±1.9 µg/mL), and were greater than drug ELF levels for both oral and IT routes demonstrating that ciprofloxacin has the capability to reach intracellular pathogens.

Formulation of ciprofloxacin – liposomal ciprofloxacin

Liposomal formulations of ciprofloxacin have been widely reported as an innovative approach to modulate the drug disposition in the lung following inhalation. Ciprofloxacin-loaded liposomes have been prepared from a number of different lipids, including egg phosphatidylcholine,

cholesterol, hydrogenated soy phosphatidylcholine and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) [131,134,135]. Two liposomal ciprofloxacin formulations, Lipoquin® and Pulmaquin® are currently undergoing phase 3 trials. HSPC and cholesterol were selected for the development of the commercial formulations due to their optimal long residence time in the lung and their availability as GMP-grade excipients [135]. Liposomes have also been associated with improved bacterial delivery as due to liposomal interactions with gram negative outer membranes facilitating antimicrobial penetration [136].

Pre-clinical studies illustrated the clear difference in absorption profiles of liposome-encapsulated and free drug from the airways. Ong and colleagues [130] demonstrated that liposomal ciprofloxacin was retained for longer within the isolated perfused rat lung model following intratracheal deposition ($t_{1/2}$~99 mins vs ~12.7 min for free drug). This was further amplified in *in vivo* studies ($t_{1/2}$ =697 min liposomal ciprofloxacin vs 7.8 min free drug). The effect on PK has also been demonstrated to translate to improved antimicrobial efficacy, particularly in the *Francisella tularensis* mouse infection model, where the longer retention time of ciprofloxacin in the lung has been demonstrated to dramatically improve survival rates compared to free ciprofloxacin [131,132].

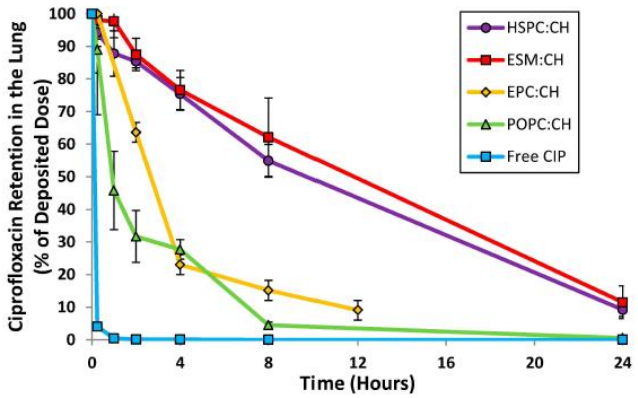


Figure 4. Effect of liposomal encapsulation in a number of different liposomal formulations containing egg phosphatidylcholine (EPC), cholesterol (CH), hydrogenated soy phosphatidylcholine (HSPC), egg sphingomyelin (ESM) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) on ciprofloxacin lung PK and comparison to non-encapsulated free drug. Figure from Cipolla et al (2016) .

A phase 1 clinical trial of Lipoquin in healthy volunteers demonstrated that the formulation was well tolerated, and that serum levels remained low following inhalation [135] . A multi-centre, Phase 2 trial in CF patients illustrated that the formulation was also efficacious in terms of significantly reducing bacterial load in sputum, and improving lung function (FEV_1).

Pulmoquin® is a combination formulation consisting of both free and liposomal ciprofloxacin together in the same product, and was developed to combine the controlled release benefits of liposomal ciprofloxacin with the rapid peak concentrations possible from free drug [135]. Pulmoquin® was tested for efficacy and tolerability as an inhaled treatment for non-CF bronchiectasis, given the promising performance of liposomal ciprofloxacin for CF infection [137]. Pulmoquin® treatment resulted in significantly decreased *Pa* sputum density (P=0.002) to day 28 compared to placebo, as well as increased time to pulmonary exacerbation. Although lung function (FEV_1) did not improve, the formulation was well-tolerated, and did not induce significant changes in ciprofloxacin susceptibility. Larger phase-3 clinical trials completed in late 2016, and

will give valuable insight into the efficacy of Pulmoquin® as a treatment option for a condition which has, as yet, seen limited treatment success with inhaled antimicrobials.

4.4.2 Levofloxacin

Molecular properties

Levofloxacin, the (S)-(-) –optical isomer of oflaxacin, is a broad-spectrum antimicrobial which has activity against gram negative and gram positive organisms associated with respiratory infection[138]. Like ciprofloxacin, it demonstrates good oral bioavailability [139] which is attributed to its physicochemical properties. Levofloxacin has relatively high aqueous solubility (~17 mg/mL) which increases as pH decreases, due to ionisation of the piperazynil and carboxyl groups [140]. The molecule also demonstrates moderate lipophilicity; the neutral species at pH 7.4 has a logP of -0.25 [140].

Relatively high concentrations have been measured in sputum following oral administration [141], however inhaled drug delivery would enable higher local concentrations to be reached whilst reducing systemic exposure to levofloxacin and associated toxicity concerns. Levofloxacin is mostly well-tolerated, however adverse effects including gastrointestinal symptoms (e.g nausea, vomiting, diarrhoea), headache, changes in taste, phototoxicity and cartilage damage have been reported [142], which would warrant a benefit to inhaled delivery, as well as reducing the GI flora exposure and risk of *C. difficile* infection.

Efficacy

Intrapulmonary delivery of levofloxacin has been demonstrated to be an effective treatment in pre-clinical models of acute and chronic lung infection with *P. aeruginosa*. In the acute and chronic infection models, levofloxacin by inhalation was associated with significantly reduced bacterial counts ($P<0.05$) than the same dose administered through the systemic route [143]. The bactericidal effect of inhaled levofloxacin was shown to be superior to that of aztreonam and tobramycin (100% survival rate for levofloxacin vs 60% tobramycin and 20% aztreonam), demonstrating impressive efficacy for this formulation.

A randomised, placebo-controlled, double-blind Phase II study in CF patients demonstrated that inhaled levofloxacin resulted in dose-dependent decrease in bacterial sputum density and improvement in lung function compared to placebo [144]. No consistent changes were observed in presence of other organisms in sputum, suggesting no selection for alternative species caused by the treatment over this time window and no change to the MIC_{50} or MIC_{90} for levofloxacin were observed.

A Phase III placebo-controlled trial of levofloxacin solution (APT-1026) in CF patients also demonstrated an improvement in lung function (FEV_1) and reduction in PA sputum density at the study endpoint (28 days) following levofloxacin treatment compared to placebo [44]. However the primary endpoint of the study, reducing the time to exacerbation, was not met. The reasons for this failure are unclear, and require further investigation as to the reasons why APT-1026 therapy has not been demonstrated to deliver this outcome.

Interestingly, in addition to the antimicrobial effects of levofloxacin, evidence of immunomodulatory effects has also been demonstrated for the compound. Exposure of human bronchial epithelial cell line HBE135 to increasing concentrations of levofloxacin resulted in decreased production of the pro-inflammatory cytokines IL-6 and IL-8, in contrast to tobramycin

which induced increased cytokine expression at the same drug concentrations [145]. These data suggest that levofloxacin may confer an additional advantage in the diseased and inflamed lung compared to other inhaled formulations on the market [146].

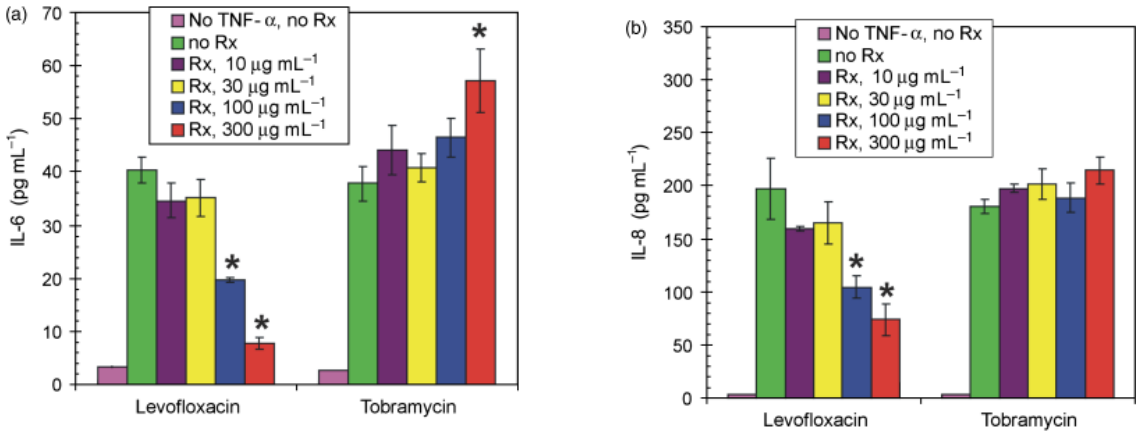


Figure 5. Effect of levofloxacin and tobramycin on (a) IL-6 and (b) IL-8 cytokine release in NL20 human bronchial epithelial cells stimulated with TNF-α following antibiotic exposure for 48 h. *P<0.005 significant difference between cells exposed to TNF-α+antibiotic and TNF-α alone. Figure from Tsivkovskii et al (2011) .

An open-label, phase III, randomized clinical study was performed to compare the efficacy of tobramycin for inhalation (TIS, TOBI®) with APT-1026 for treatment of chronic *PA* infection [147]. The primary endpoint of the study was met, demonstrating non-inferiority of levofloxacin solution to TOBI® therapy in terms of improvement to FEV_1, however no significant superiority of levofloxacin treatment was identified. A follow on study consisting of three-further cycles of APT-1026 administered to patients from either the LEVO or TOBI® treatment groups has been recently reported [148]. A majority of patients who switched from TOBI® to levofloxacin therapy showed an improvement in their % predicted FEV1 above their post-TOBI® baselines following treatment cycles 4 (77.4%), 5 (78.6%) and 6 (72%), which demonstrates the potential benefit of switching antibiotic regimes during prolonged treatment. Long term levofloxacin therapy (LEV/LEV treatment group) was demonstrated to maintain lung function over the 48 week study period, indicating that the therapy was able to halt the rate of lung function decline expected of a CF patient over this time window. However, whilst levofloxacin has been demonstrated as a non-inferior treatment option for chronic infection in CF, at present its superiority to other treatment options has not been demonstrated.

Pharmacokinetics of inhaled levofloxacin

The isolated perfused rat lung model of lung absorption has as shown that there was limited presence of levofloxacin in the efferent perfusate fluid and higher concentrations in lung tissue following instillation in the lung compared to systemic administration [149]. In a mouse model, lung homogenate samples following intrapulmonary delivery had a 9-fold higher AUC value and 30-fold higher C_{max} value than systemic administration, showing clear improvement in drug targeting to the airways with localised delivery [143].

In human CF patients, inhaled levofloxacin (MP-376) resulted in high sputum concentrations and low systemic drug levels, however serum T_{max} of levofloxacin (median values 0.17 – 0.25h)

suggested rapid absorption from the lung following inhalation, in contrast to some of the more hydrophilic classes discussed in this review [43].

Formulation of levofloxacin for inhalation

Aeroquin® (Forest Laboratories, USA) and Quinsair® (Horizon Pharma Europe BV) are both levofloxacin solutions which have been specifically developed for use in the lung. Levofloxacin solution for inhalation has been designed to incorporate divalent cations and permeant ions [143] which have been identified as highly important for the tolerability of inhaled formulations [150]. Levofloxacin solution has been demonstrated to be well tolerated in a number of clinical studies, with limited treatment-associated adverse events. The most commonly reported adverse events include complaints about taste and cough [43,144].

Formulation of levofloxacin for inhalation – dry powder formulations

Formulation developments for levofloxacin have focused on reducing treatment burden and using controlled release formulations to modify the drug's fate in the lung. Akdag Cayli et al [151] have recently reported the preparation of spray-dried combination levofloxacin/N-acetylcysteine (NAC)or DNase powders. The powders demonstrated good aerodynamic properties, showing potential to reach the deep lung upon inhalation. Levofloxacin delivered as dry powder demonstrated lower permeability across Calu-3 cells grown at the air-liquid interface compared to the same dose in solution; however levofloxacin still demonstrated higher permeability than ciprofloxacin illustrating its ability to rapidly cross epithelial barriers.

The rapid absorption profile of levofloxacin means that there is considerable interest in developing controlled release formulations for the drug in the lung. As absorption is not a barrier to lung transport, levofloxacin levels could be sustained for longer in the airways by providing a reservoir for gradual release following inhalation. Poly(lactide-co-glycolide) (PLGA) is a biocompatible, biodegradable polymer which has been demonstrated to be suitable for preparation of controlled release carriers for use in the lung [152]. PLGA microspheres (MS) have been used to encapsulate levofloxacin for inhalation, where the controlled release formulation has demonstrated significant effects on PK parameters [153]. Delivery of PLGA-MS-levofloxacin to the rat lung resulted in a dramatic increase in the ELF to plasma ratio (ELF-to-plasma total $AUC_{0.5-t}$), from 1.21 for aerosolized solution to 169 for aerosolized PLGA-MS levofloxacin powder. Plasma concentrations also decreased more slowly compared to free drug, indicating a slow release of drug from the carriers following deposition. The tolerability of the formulation and the fate of the carriers after drug release have not yet been investigated. If this formulation proves to be well-tolerated, it will be of great interest to investigate if the sustained drug levels in the lung can translate to increased antimicrobial efficacy in pre-clinical and clinical respiratory infections. As fluoroquinolones exhibit concentration-dependent activity [10], formulation approaches which increase the concentration at the site of infection for longer would be hypothesised to be highly valuable in increasing the efficacy of levofloxacin inhaled treatment.

5. Future directions

5.1 Combination therapies

Combination therapy is of increasing interest due to its ability to slow or even stop the increase in antimicrobial resistance following antimicrobial therapy. The success of combination therapies is due to the reduced possibility that an individual organism will possess AMR genes which protect against both antimicrobial agents administered [154]. When the administration of two antimicrobial agents results in greater efficacy than would have been expected from the sum of the individual drug's activities, the agents can be described as synergistic [155]. The improved antimicrobial efficacy is quantified by the sustained MIC levels which are observed following prolonged combination therapy, compared to increasing MICs if a single agent is repeatedly administered[154].

In pulmonary antimicrobial therapy, combination therapies have been developed by the design of combination-drug dry powders suitable for inhalation. A combination spray-dried ciprofloxacin and gatifloxacin powder (28:72 w/w) has been reported [156] which was demonstrated to have synergistic activity *in vitro* against *Pa* compared to single drug powders. By contrast, ternary powders of ciprofloxacin, gatifloxacin and lysozyme demonstrated no synergistic effect. Further investigation of co-formulated spray-dried ciprofloxacin combination powders has identified that a ciprofloxacin /gatifloxacin /ambroxol powder (formulated with L-leucine) demonstrated an improvement in synergistic formulation with increased antimicrobial efficacy against *Pa* than the binary mixture [157]. Combination powders of spray-dried colistin/crystalline rifapentine [158] and ternary combinations of colistin/meropenem/tigecycline/rifampicin (3 drugs per powder) have demonstrated synergistic activities in *in vitro* bacterial models [159]. Translation into pre-clinical studies will be of great interest to identify the efficacy of the treatment in the living host.

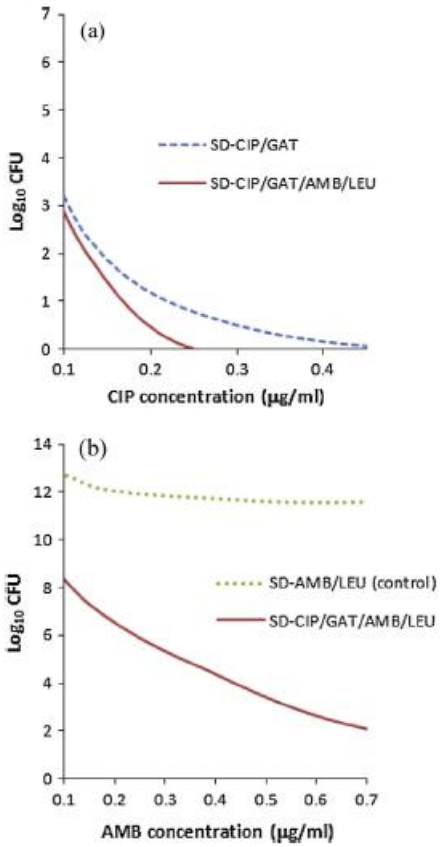


Figure 6. Comparison of dose response kill curve for combination spray-dried powders ternary ciprofloxacin HCl/ gatifloxacin HCL/ ambroxol powder with L-leucine (SD-CIP/GAT/AMB/LEU), binary ciprofloxacin HCl/gatifloxacin HCl (SD-CIP/GAT) powder and ambroxol/leucine (SD-AMB/LEU) control. Improved antimicrobial activity was demonstrated by the combination powders with SD-CIP/GAT/AMB/Leu> SD-CIP-GAT > SD-AMB-LEU. Figure from Huey-Lee *et al* (2015) .

Inhaled aerosolised combination therapies are already currently in clinical trials. A Phase 1 study of combination amikacin-fosfomycin therapy administered in aerosolised solution to 20 mechanically ventilated patients with ventilator-associated tracheobronchitis (VAT) or ventilator-associated pneumonia (VAP) demonstrated that the therapy resulted in high levels of both amikacin and fosfomycin in tracheal aspirates, with far lower plasma levels than would be expected for intravenous therapy [160]. A Phase 2 study in 143 patients found that although combination amikacin/fosfomycin treatment reduced bacterial load in tracheal cultures, overall clinical outcomes were not improved [161]. Combination amikacin/fosfomycin has been demonstrated to have high potency against important respiratory pathogens (*P. aeruginosa, K. pneumonia*) in *in vitro* assays [162], and so the mechanisms of why this has not translated to improved clinical outcomes will be of great interest.

5.2 Engineered microparticles

This review has described a number of approaches to produce engineered microparticles for dry powder formulations. However, some additional micron-scale carriers for use in inhalation not yet in the stage of clinical development nevertheless give an interesting insight into further possibilities to modify antimicrobial disposition in the lung through formulation means.

The fast absorption of ciprofloxacin after inhalation leads to shorter retention time in the lung, and thus difficulty in sustaining high drug concentrations. Tewes et al [163] have reported a novel solution employing ciprofloxacin-calcium composite microparticles, in which the presence of calcium reduced the apparent permeability of ciprofloxacin through a model of the respiratory epithelium up to ~84%, whilst retaining antimicrobial activity against *Pa* and *Staphylococcus aureus*. The formulation has not yet been tested *in vivo* but this initial study indicates an important role to be played by inorganic excipients in modulating antimicrobial molecule PK following administration to the lung.

Niosomes are multilamellar non-ionic surfactant vesicles which can be used to encapsulate hydrophilic and hydrophobic drug molecules, and can be easier to store, purify and handle than liposome formulations [164].Ciprofloxacin-loaded niosomes prepared from Cholesterol, Tween 60 or 40, and Span 60 or 40 (diameter d_{50} ~3.7 – 4.3μ m) were demonstrated to have improved activity compared to free ciprofloxacin against laboratory isolates of *K. pneumoniae* and *P. aeruginosa* and two clinical isolates of *Pa*, including a clinically resistant strain [165]. This observation was in line with previous findings demonstrating the improved efficacy of ciprofloxacin against gram negative bacteria when encapsulated in liposomes [131,132]. Ciprofloxacin-niosomes could be aerosolised via nebulisation and demonstrated a high FPF (~50-62 %). The carrier was well tolerated, with the loaded niosomes having a lower IC_{50} than ciprofloxacin alone. The formulation requires improvement to stability and retention of drug following nebulisation, however niosomes represent a promising formulation for delivery of antimicrobials for gram negative respiratory infection.

5.3 Nanoparticle carriers

Nanoparticles have long been investigated for their potential use as drug carriers in the lung because of their ability to modulate release kinetics and improve drug targeting [166]. In antimicrobial delivery, nanoparticle carriers can provide protection for therapeutics from the harsh environment of the infected lung [167], modify the PK of inhaled therapies [168] and improve intracellular drug delivery of antimicrobials, which would be of benefit in treatment of intracellular respiratory pathogens including *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Legionella pneumophila [169]*.

A number of different materials have been employed for inhaled antimicrobial nanocarriers which have been investigated in *in vitro* and *in vivo* models of respiratory cells and tissues and relevant respiratory pathogen cultures. Gunday-Tureli and colleagues [170] have recently reported the use of polymeric nanoparticles (PLGA) to encapsulate ciprofloxacin (CIP) in complex with sodium dodecyl sulphate (SDS), which the authors postulate can increase antimicrobial activity by reducing efflux transport of ciprofloxacin. Drug release from the PLGA-SDS-CIP formulation demonstrated a controlled release profile (<30% being released by 1 h). PLGA-CIP-SDS nanoparticles increased antimicrobial activity against *Pa* compared to free CIP as

determined by inhibition zone assay. D'Angelo et al [167] have reported the preparation of polymeric nanoparticle carriers for the delivery of colistin to the lung, to protect colistin from inactivation in the respiratory extracellular environment, as well as prolonging drug concentrations in the lung following inhalation. Colistin-loaded chitosan (CT) and poly(vinyl-acetate) (PVA) nanoparticles were prepared of ~300 nm in size with encapsulation efficiencies of >60%. Encapsulation resulted in a slow release of drug, which was markedly slower for PVA particles than for chitosan (~50% of colistin released from CT-NP after 5h vs ~5% from PVA NP). Both formulations could permeate through an artificial CF mucus model, with greater penetration for CT-NP. Nanoparticles were engineered into a dry powder for inhalation by spray-drying with lactose to form nano-embedded microspheres (NEM). All formulations were demonstrated to penetrate *Pa* biofilms, showing targeting ability to organisms within biofilm. PVA-NEM showed a weaker biofilm activity than free drug at 24 h, however activity was preserved over 72h. CT-NEM demonstrated less biofilm activity than the two other formulations tested.

Lipid-based nanocarriers have received much interest for inhaled drug delivery. They are versatile carriers, being suitable for both hydrophilic and hydrophobic molecules, and being prepared from naturally occurring materials, they may be able to exploit endogenous breakdown and clearance mechanisms to reduce concerns about long term particle accumulation upon repeat dosing [171]. Nanostructure lipid carriers, comprised of a solid lipid core surrounded by a surfactant shell, have been used to encapsulate tobramycin (>90% encapsulation efficiency) resulting in enhanced penetration through an artificial mucus model compared to free drug [172]. Antimicrobial efficacy was maintained or increased following lipid encapsulation, were well tolerated by respiratory cell models and demonstrated a long lifetime in the lung following instillation (>48 h). Lipid nanocarriers of sodium colistimethate have also been reported to improve antimicrobial efficacy compared to free drug, with a greater number of *Pa* clinical isolates had MIC <1µg/mL for NLC-colistimethate compared to free drug [168]. The carriers also demonstrate good tolerability (IC_{50} NLC-colistimethate 28-160 fold less than the free drug in two respiratory cell culture models, H441 and A549) and long retention time in the lung (>48h) showing potential as a controlled release carrier for this chemical class in the lung. Demonstration of *in vivo* antimicrobial efficacy would be highly beneficial in order to determine the clinical usefulness of this technology to improve inhaled infection treatment.

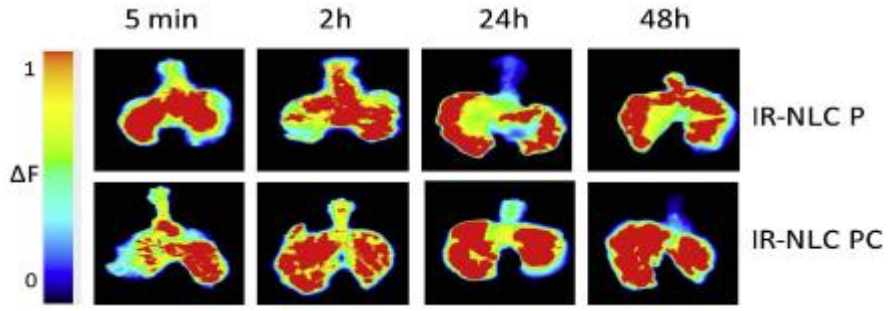


Figure7. Lung distribution of IR-783-labelled non-drug loaded (IR-NLC P) and ciprofloxacin-loaded (IR-NLC PC) nanostructured lipid over 48 h post intratracheal instillation in the mouse lung. Figure from Moreno-Sastre *et al* (2016).

Other interesting nanocarrier systems recently reported for respiratory use include polymer-lipid-glyceromes [173] and mucus-penetrating polymer-drug crystals [174], both of which can modulate drug disposition following inhalation and offer exciting, formulation-based approaches to overcome challenges associated with delivery of antimicrobial molecules to the lung.

5.4 Emerging therapeutics

With the increasing incidence of antimicrobial resistance, there is a desperate need for new antimicrobial therapies, particularly those with broad spectrum activity or against the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter*) pathogens, however the antimicrobial development pipeline has slowed to a bare minimum [175]. When new compounds are identified, administration is usually first considered via either oral or intravenous routes depending on disease severity. However, with the increasing incidence of antimicrobial resistance in respiratory pathogens, and the severity and morbidity of respiratory infection, should we pay more attention to the inhaled route for new emerging antimicrobial compounds?

This review has highlighted the significant success of inhaled antimicrobial therapy for respiratory infection, particularly in cystic fibrosis where it is now regularly part of the treatment regime [7]. As new antimicrobial compounds emerge, the inhaled route could offer a highly effective strategy to administer compounds directly to the site of respiratory infection, lowering unnecessary systemic exposure and increasing the speed and efficacy of infection treatment. In addition, by reducing systemic drug levels, the exposure of commensal bacteria in the gastrointestinal tract to desperately needed new antibiotics is reduced. This can assist in the responsible stewardship of new drug, helping to reduce the emergence of AMR-organisms and in turn reducing the risk of AMR-pathogens spreading throughout the community from host to host, or within the healthcare setting from patient to patient.

A challenge for any new antimicrobial compound form inhalation will be ensuring that the molecular properties allow effective treatment of respiratory infection with a tolerable dose-range and without detrimental effect on the host. As explored in this review, a number of these properties can be modulated with the use of formulation technologies, which could be applied when formulating new antimicrobials for the lung.

With the use of molecular modelling techniques to identify ligands and improve molecular interactions, compounds emerging from the development pipeline are likely to increase in hydrophobicity [176]. This will increase the difficulty in sustaining high concentrations of drug within the airways, due to the increased permeation through the respiratory epithelium with increasing logP [30]. Encapsulation in liposomes or nanoparticle-based carriers can be employed to provide controlled release reservoirs of the antimicrobial, gradually releasing drug following inhalation to allow for a sustained high drug concentration in the lung. As discussed previously, liposomal carriers can enhance penetration of antimicrobials into gram negative bacteria [136], potentiating the effects of antibiotic molecules as well as affecting their PK. With increased hydrophobicity, achieving high drug doses in the lung can become challenging. Nanoparticle, niosome and liposome-based carriers are all capable of encapsulating hydrophobic drugs, and can offer a means to achieve high therapeutic payloads in the lung despite limited aqueous solubility.

Inhaled drug delivery can also be beneficial in reducing concerns about systemic side effects of new antimicrobial molecules. As demonstrated across the antimicrobial classes covered in this review, it consistently results in reduced plasma concentrations compared to the systemic routes

of administration, allowing targeted delivery straight to the site of infection and minimising exposure to vulnerable organs and tissues such as the kidney, inner ear and joints. Thus inhalation can be a safer route of administration to reduce these risks.

Finally, the administration route is less invasive, and more convenient for the patient. Particle engineering technology has enabled the design of highly effective handheld drug-device combinations, capable of delivering high payloads of drug to the infected lung in a matter of minutes. In comparison to intravenous therapy, inhalation can offer a simple and fast route to antibiotic administration which does not require the use of sterile needles or trained medical professionals. Although it would not be suitable for the current antimicrobials on the market, it is worth considering that due to its large surface area and small barrier to absorption [177] in the advent of a small, lipophilic and highly potent antimicrobial being identified, the inhaled route could even provide a non-invasive means to deliver a drug to the systemic circulation. The flexibility and importance of the administration route should not be underestimated, and should be part of the consideration when developing formulations for any newly identified antibiotic.

6. Conclusions

Respiratory infections affect millions of people worldwide, contributing massively to mortality worldwide. As antimicrobial resistance increases, these infections are becoming increasingly challenging to treat and require ever more sophisticated strategies both to effectively end infection and also to minimise the spread of antimicrobial resistance.

Inhaled drug delivery has been profoundly effective in the treatment of respiratory infection using a diverse range of antimicrobial chemical classes, from small lipophilic drugs (ciprofloxacin, levofloxacin), to charged hydrophilic compounds (amikacin, tobramycin) and even antimicrobial peptides (colistin). It has enabled to delivery of high concentrations of therapeutic directly to the site of infectious organisms, bypassing the need for high systemic doses and reducing to a bare minimum the number of cellular barriers which must be traversed by the molecule before reaching the bacterial organism. For these reasons, it has been associated with significant improvements in antimicrobial efficacy (as quantified through sputum bacterial density) and lung function. It has now become a vital and valued part of treatment for chronic *Pa* infection in CF patients, and is likely to be incorporated into the treatment of many other respiratory infections in coming years.

Formulation approaches have been instrumental in optimising drug disposition following inhalation. Liposomal formulation and particle engineering technologies in particular have been immensely valuable in enabling modification of drug PK and reducing the treatment burden of inhaled therapies, respectively. As medicinal chemists strive to identify desperately needed novel antimicrobial compounds, pulmonary drug delivery should be considered when developing the compound into formulations for delivery. For antimicrobials, the inhaled route is an attractive option to maximise drug efficacy, whilst limiting necessary systemic exposure to both to host tissues and commensalism flora. It is time to take the inhaled route seriously when considering how to use novel antibiotics most efficiently and responsibly to treat respiratory infection.

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